north before the advance of the glacial climate might be forced during its advance to migrate southward to maintain their existence, and on the retreat of the same might climb up the mountain sides and remain there as witnesses of their former migration. Moreover, plants which formerly had been spring blossomers might during the advance of glacial climate maintain themselves *in situ*, by adapting themselves to the more rigorous climate. On the retreat of the glacial conditions they might have so altered their habits as to be able to maintain their existence only on mountain tops or in the distant north. Migration to these places would therefore set in. It is probable that all these causes have operated in the production of spring blossoming plants. It is impossible to tell in the case of individual plants, to which method their production is to be ascribed. It is sufficient for the present to remember that nature has many means of accomplishing the same result.

Heidelberg, Germany.

The effect of mechanical movement upon the growth of certain lower organisms.

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The effect of external agencies upon the growth of organisms has been thoroughly studied in several of its relations. Of these influences, the relation of temperature to growth, is perhaps the best understood. Other factors, such as the effect of light, of increased and diminished pressure, have also been made the subject of more or less careful study.

To the effect of mechanical movement upon the growth, less attention has been given, nor have the results already obtained been entirely in harmony with one another.

In the following experiments an attempt has been made to find out, (1) what influence mechanical movement has upon growth of cells in regard to size and form; (2) its influence upon growth in regard to increase in number.

The method used in the experiments was as follows:

Two 500 c. c. distilling flasks were half filled with nutrient solutions, sterilized and then inoculated with a small quantity of the germ to be studied. After thoroughly distributing the

inoculated "seed" by shaking, a number of samples were taken and from these the number of cells in a certain volume was determined by means of Nachet's hæmacytometer. By means of this apparatus equal known quantities were used each time. A number of counts, usually not less than one hundred, were made, so as to make the average as accurate as possible. At first each flask was inoculated and counted separately, but it was soon found that more accurate results could be secured by determining the number present per unit of volume for the whole amount of the fluid and then dividing it into two equal amounts. The danger of contamination from outside influences is of less consequence than the difference in units of volume which inevitably occurs where the determinations are made separately of the amount of "seed" added to each flask. After counting, the two flasks were subjected to exactly the same conditions, with the exception that one of them was kept in a state of constant agitation.

This was secured by the rotation of a vertical shaft to which was attached a horizontal bar. This bar in its rotation struck and lifted the flask, which on its return swing struck against an upright standard. The stroke by the rotating bar and the sudden checking of the movement of the flask kept the fluid in a state of constant agitation. All points of contact of the flask with the bar and standard were sheathed with rubber to prevent breaking. The power was furnished by a small reaction water wheel and was transmitted by a belt from a pulley on the motor to a cone pulley fastened to the upright revolving shaft. This cone pulley enabled me to vary the rapidity of the revolution of the horizontal arm at pleasure.

After allowing a certain time for growth, samples were withdrawn and counted in the same way as before. The number per unit of volume at the close of the experiment divided by the number per unit of volume inoculated as "seed" gives the ratio of increase for each flask. As a check, the solution was sometimes filtered and the organic substance carefully collected, dried, weighed, and the ratio thus determined. For this, the ash constituent of the cells can be neglected without interfering materially with the accuracy of the results.

In the majority of the experiments, *Monilia candida*, a yeast-like germ capable of inducing alcoholic fermentation in most sugar solutions, was used for the tests. Two other

germs, *Oidium albicans* and *Saccharomyces mycoderma*, were also used in various cases to confirm results.

To determine the effect of movement upon the size and form of the cells, an experiment was made with Oidium albicans in a bouillon culture. This germ when grown in this culture medium in the ordinary way forms two types of cells, one of which is a long, slender, hypha-like filament, and the other, a short, oval, or oblong yeast-like cell. As a rule, the growth mass is more or less gelatinous, the jelly-like consistency being due to the intimate intermingling of the elongated cell type. Where the short type of cells prevail, the vegetative mass in the bottom of the flask is of a more sandy nature. Two flasks were inoculated with this germ and subjected to similiar conditions except that one of them was kept in a state of constant movement while the other was left undisturbed.

The results obtained were as follows: in the still flask, the two types of cell structure were present in the usual proportions, and a wide variation was found in the yeast-like cells. These varied from 4×8 μ wide to 7×14 μ long. In the shaken flask no true hyphal filaments were found, such as were present in ordinary cultures. The nearest approach to true filaments were several chains of 4–6 slightly lengthened cells. The cell-contents did not differ materially in samples taken from each flask.

Much less variation in size was found among the cells of the shaken flask than in the other one.

The experiment was repeated with Saccharomyces mycoderma, a mycelium-building yeast. With this germ, the time of incubation was extended until quite a thick veil or membrane had formed over the surface. The still flask showed two types; one a submerged form, slender in outline, about $0.85\times2.25\,\mu$, and the other a surface form with vacuolated contents and plumper outline, averaging $1.2\times2.5\,\mu$. In the flask which was kept in motion there was a greater variation found in size, but that was owing to the large number of young daughter cells that had broken away from the mother cells before maturity. The mature cells were quite uniform in size, broadly oval in form, and almost all highly vacuolated. The average size was $1.25\times2.25\,\mu$.

From the above experiments it would seem, first, that incessant movement tends to prevent the formation of true hyphal filaments, although elongated types of cells are found;

second, that with the germs forming torula-like cells but little difference in size and shape can be produced. What difference there is seems to be caused not so much by the agitation of the fluid as by the exposure of the cells more freely to the influence of the atmosphere. When submerged the cells grow slender, with homogeneous contents, while the surface-grown forms were highly vacuolated and more broadly oval. The cells from the shaken flask agree in all essentials with those grown at the surface of the still flask, except that they averaged somewhat larger in size.

The second series of experiments bears upon the influence of mechanical movement upon the increase of cells. Horvath[1] conducted a series of experiments upon bacteria and their relation to movement, in which he found that mechanical movement interfered materially with the growth of the germs. From this he made the sweeping generalization that movement had a retarding influence on the growth of all lower organisms. Hansen[2] investigated the subject in connection with his work on yeast (*Saccharomyces cerevisiæ*) and found the reverse to be true. The germs increased two to three times faster when agitated than they did when grown at rest. He concluded that it was the agitation of the cell itself, aided possibly by the more minute subdivision of the nutritive materials, that enabled it to increase more rapidly. The introduction of air into the fluid by the apparatus he used was so little that he thought this point was not of much importance.[3]

In the following synopsis of results, A in all cases represents the culture agitated and B the one that grew undisturbed.

NO. OF EXP.	KIND OF GERM.	HOURS OF GROWTH.	NO. OF GERMS PER UNIT OF VOLUME.		PROPORTION SHOWING RELATIVE INCREASE IN NO. OF CELLS.	RATIO BETWEEN A AND B.
			BEFORE EXP.	AFTER EXP.		
A I. B I.	Monilia candida.	94	16.9+	2332 1618	1:138 1: 95	1.45+
A II. B II.	Oidium albicans.	48	4.67+	1262 610	1:270 1:130	2.0+
A III. B III.	Monilia candida.	70	24.4+	1087 767	1: 44+ 1: 31+	1.41+

[1] Horvath: Pfluger's Archiv f. d. ges. Phys. XVII, 125.
[2] Hansen: Medd. fra Carls. Lab., I, 271.
[3] Hansen: Hypothèse de Horvath, Medd. f. Carls. Lab., I, 96, French résumé.

The culture solution used in all cases was a 10 per cent. solution of grape sugar to which 1 per cent. peptone had been added. The above results indicate without exception that those germs which were agitated increased from 1.4–2 times as fast as those grown undisturbed. As a check upon the counting process, determinations of the dry matter present were made by chemical analysis at first.

Exp. III, which showed a ratio between A and B of 1.41 by the counting process gave by chemical analysis the following result. Amount of organic matter formed in A_{III} 0.1778 gm.; in B_{III} 0.1293 gm. Ratio between A and B 1.37+. This proves that the counting process is reasonably exact as it agrees quite closely with the chemical analysis. This is only true however where there is general uniformity in size of the cells.

The amount of alcohol which was produced by this germ when subjected to these different conditions, was also determined in a number of cases. In every case where this was made, a considerable increase in amount of alcohol formed was found in the undisturbed culture (B) over the agitated culture (A). It would seem then that agitation exerts a favorable influence upon the formation of cells but a retarding effect upon the products of fermentation. Both of these processes, growth and fermentation, depend directly upon the kinetic energy of the plant organism. Where katabolic processes are manifested more strongly in fermentative action there seems to be less energy used by the plant in growth. The data of the two following experiments with Monilia candida, giving the highest and lowest proportions found by analyses, illustrate this point.

NO. OF EXP.	ALCOHOL FORMED.	INCREASED GROWTH OF SINGLE CELL.	RATIO.
A_V	1.6%	335	
B_V	3.2%	109	
Proportion.	1 : 2 :: 3.07 : 1		6.14
A_{VI}	3.2%	44	
B_{VI}	3.8%	31	
Proportion.	1 : 1.18 :: 1.41 : 1		1.66

It will be noted that while no uniformity seems to exist in the ratio, the *amount* of *fermentation products* of the cells in B is *always* greater than in A, while the *amount of organic matter* formed stands in an inverse relation.

We may now ask what is the cause of this increased rapidity of growth when agitated. The experiments detailed above allowed considerable aeration during the movement and as this factor seemed most prominent, the experiments were repeated in such a way as to increase the aeration and diminish as much as possible the movement of the fluid. If aeration increases the growth of the organisms, there should be an increase in the ratio between A and B.

Exp. I. An Esmarch's coiled glass tube, such as is used in bacteriology for air determination in fluid cultures, was inoculated with Monilia candida and air drawn slowly through the coil by the aid of an aspirator. The small bubbles of filtered air slowly travel the spiral, so that a considerable quantity of oxygen ought to be absorbed by the liquid. In this way aeration is considerably increased while the movement of the fluid is much reduced. At the end of 42 hours growth, it was found that the germs which were aerated had increased 2.5 times as fast as the non-aerated culture.

Exp. II. A 500 c.c flask was partially filled with a nutrient solution and inoculated with freshly grown Monilia candida. The mouth of the flask was closed by a triple-perforated sterilized rubber cork. In two of the openings, glass tubes were inserted and the lower ends were drawn out into fine points. The third opening was closed by a bent open tube, the outer end of which was directed downwards. These glass tubes were closed with cotton-plugs and sterilized before being put in place. The two capillary tubes were connected to the blast of a filter pump and thus a stream of filtered air was forced into the fluid culture. The fine bubbles of air rising to the top of the fluid escaped through the bent exit tube. In 30 hours there was found to be 2.2 times as many cells per unit of volume in aerated flask as there were in non-aerated.

Exp. III. In both of the preceding experiments aeration was increased as much as possible while the movement was lessened. In this experiment the reverse order was followed. A thick glass tube was sealed at one end and at 5 cm. distance from this end, a large bulb capable of holding 300 cc.

was blown. In this culture bulb was placed some coarse sterilized quartz sand. The longer open arm was closed with cotton. The vessel was filled with culture fluid so that the bulb and a portion of the open arm was entirely filled. This apparatus was connected with the motor and so arranged that it revolved in as nearly a horizontal position as possible. The coarse sand inside acted as a distributor of the motion to the fluid causing it to be agitated thoroughly.

In this way the maximum movement was obtained with a minimum of aeration; the only chance for aeration being through the small opening of the open arm. Cultures of Monilia candida grown for forty hours and treated in this way had 1.4 times as many cells as those grown in undisturbed flasks.

It is practically impossible to get a considerable movement of the liquid without more or less aeration and the converse is equally true, but where aeration was increased in greater proportion than movement, as in Exp. I and II, we find the percentage of increase of cells and consequently of organic material to be much greater than in Exp. III, where aeration was diminished relatively more than movement.

This factor of aeration seems to be the predominant one although it is possible that the increase is not due to the action of any one factor alone. More intimate division of nutritive materials and the constant presentation of fresh food material to the surface of the plant cell probably aids in the increased growth.

Summing up the points discussed into concluding sentences, we may say that:

1. The form and size of fungal cells is but little influenced except in the case of hyphal filaments which seem to form with difficulty when subject to constant movement.

2. Constant agitation affects very strongly the increase in number of cells formed and consequently the amount of organic matter produced. The increase by growth in agitated cultures as compared with still-grown cultures ranges between wide limits but is usually 200–300 per cent.

3. The amount of fermentation products, as determined by the alcohol formed, seems to stand in an inverse ratio. All cultures so tested showed uniformly less alcohol in agitated than still cultures.

4. The cause of this more rapid cell-multiplication by mechanical movement seems to depend upon aeration of the

culture, the cells growing more rapidly in contact with atmospheric oxygen than when submerged.

5. While this appears to be the chief factor, other elements such as better conditions of nutrition, etc., probably enter in as less important factors.

These researches were carried on in the biological laboratories of the University of Wisconsin.

Baltimore, Md.

Noteworthy anatomical and physiological researches.

Apical areas in seed plants.

The copious researches of MM. Van Tieghem and Douliot[1] on the origin of endogenous members in the vascular plants, published in the *Annales des Sciences Naturelles Botanique* during 1888, will be remembered by all students of contemporary botanical literature. The conclusions arrived at regarding the apical cells of monocotyledons and the single apical cell of the Archispermæ (gymosperms) are well known, having already found their way into at least one of the more prominent text-books. It is by no means universally admitted, however, that the proof of apical cells in these groups of plants is decisive. The older literature on the subject was given in résumé by Dingler[2] in 1882, but since that time the important works of Karsten,[3] DeKlercher,[4] Groom,[5] Korschelt,[6] and others have appeared, supplementing the classic researches of Strasburger, Hanstein, Hofmeister, and the rest. In the *Ann. des Sciences Nat. Botanique*, 1890, Douliot[7] reviews the later works and, adding some investigations of his own, maintains the positions advanced in 1888 in his paper in conjunction with Van Tieghem. In brief, his conclusions are as follows:

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[1]Recherches comparatives sur l'origines des membres endogènes, Ann. Sci. Nat. Botan., VII. VIII. 1. (1888.)

[2]Ueber das Scheitelwachsthum des Gymnospermen-Stammes, München, 1882.

[3]Ueber die Anlage seitlicher Organe bei den Pflanzen, Leipzig, 1886.

[4]Sur l'anatomie et le développement du Ceratophyllum, Bihang, k. Sv. Vet. Acad. Hand. IX, Stockholm, 1885.

[5]Ueber den Vegetationspunkt der Phanerogamen, Ber. der deutsch. bot. Gesell. 1885.

[6]Zur Frage über das Scheitelwachsthums bei den Phanerogamen, Pringsh. Jahrb. wiss. Bot. 1884.

[7]Sur la croissance terminale de la tige, Ann. Sci. Nat. Botan. VII, XI. 283.